ACMG Classification Rules Specified for *ATM*

Release Notes/Changes from v1: Corrected combining rules for LP to include PVS1 + PM2_Supporting = LP

Green writing is HBOP VCEP Expert opinion on *ATM* Rules

MONDO:0016419 (hereditary breast cancer) MONDO:0008840 (Ataxia-Telangiectasia) MONDO:0018266 (Ataxia-Telangiectasia, Variant)	**RefSeq: NM_000051.3 Ensembl: ENST00000278616.8**	
PATHOGENIC CRITERIA		
Criteria	**Criteria Description**	**Specification**
VERY STRONG CRITERIA		

PVS1_Variable
PVS1_O_Variable
	Null variant in a gene where loss of function is a known mechanism of disease.
	• Per ***ATM* Exon Map and *ATM* PVS1 Guide**
	  ○ **PVS1: Predicted splice defect**
	  ○ **PVS1_O: Observed splice defect**
		Gene-Specific

STRONG CRITERIA		
PS1 PS1_RNA (use as moderate)	Same amino acid or splice change as a previously established pathogenic variant regardless of nucleotide change. • **Protein**: Use only when there is no expectation of a splice defect for either variant • **RNA (use as PS1_RNA_Moderate)**: Can be applied to a variant that is predicted similar or worse than another variant with an observed deleterious splice defect. ○ Close match alterations must be approved LP/P by the HBOP VCEP.	General
PS2	*De novo* (paternity confirmed) in a patient with the disease and no family history. • Do not use for AD or AR disease: Informative *de novo* occurrences have not yet been observed and *de novo* AR conditions are unlikely to be informed by phase	N/A
PS3_Variable	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect • **Protein**: Per detailed notes section regarding rescue and radiosensitivity assays • **RNA**: Use code PVS1_O_Variable	Gene-Specific
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. • Case-control studies; p-value ≤.05 and OR ≥2 or lower 95% CI ≥1.5 (typically necessitates very large studies and/or power analysis)	Disease-Specific

Related publication(s): Date Approved: January 19, 2022

MODERATE CRITERIA		
PM1	Located in a mutational hot spot and/or critical and well-established functional domain. • Do not use: Benign and pathogenic variants are known to occur within the same domains and germline mutational hotspots are not well defined at this time	N/A
PM2 (use as supporting)	Absent/rare from controls in an ethnically-matched cohort population sample. • Rare is considered ≤.001% in all sub-populations where N is >1. • Is not considered a conflicting piece of evidence for variants that otherwise are likely benign/benign • Use as **PM2_Supporting** (not moderate)	Gene-Specific: Strength
PM3_Variable	For recessive disorders, detected in *trans* with a pathogenic variant. • Per **Ataxia Telangiectasia PM3|BP2 table**	Disease-Specific: Strength
PM4	Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. • Do not use for in frame insertions and deletions as no data are available for this rule at this time • PM4 **can** be used for stop-loss variants.	Gene-specific
PM5	Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before. • Do not use: Multiple amino acid substitutions at the same residue can be pathogenic or benign and bioinformatic tools cannot yet confidently distinguish them • Apply as **PM5_Supporting** to frameshifting or truncating variants with premature termination codons upstream of p.Arg3047 which are expected to be more severe than the most C-terminal pathogenic variant p.Arg3047*	Gene-specific
PM6	Confirmed de novo without confirmation of paternity and maternity. • Do not use for AD or AR disease: Informative *de novo* occurrences have not yet been observed and *de novo* AR conditions are unlikely to be informed by phase	N/A
PS4_Moderate	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls-Proband Counting • Do not use: Proband counting for genes causing a common disorder need to be	N/A

	calibrated in a population-specific way before use	
SUPPORTING CRITERIA		

PP1	Co-segregation with disease in multiple affected family members
	• Do not use:
	  • AD Condition: Co-segregation analysis in lower-penetrance genes can lead to false positive results (PMID 32773770)
	  • AR Condition: informative instances of co-segregation in A-T families are too rare to be considered for weight at this time. See notes section for suggested approach
		Gene-Specific

PP2	Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease. • Do not use: ATM does not have a defined low rate of missense pathogenic variation	N/A
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product • Protein: REVEL >.733 PP3 • RNA: multiple in silico predictors agree to a similar impact on splicing ○ Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.	General
PP4	Phenotype specific for disease with single genetic etiology. • Autosomal Dominant: do not use-Breast cancer is very common with a high degree of genetic heterogeneity • Autosomal Recessive: do not use as a separate line of evidence. Such evidence is built into the **Ataxia Telangiectasia PM3|BP2 table**	N/A
PP5	Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation	N/A-discontinued

BENIGN CRITERIA		
Criteria	**Criteria Description**	**Specification**
STAND ALONE CRITERIA		
BA1	GnomAD **Filtering Allele Frequency** Allele frequency • **>0.5%**	Gene/Disease-Specific
STRONG CRITERIA		
BS1	GnomAD **Filtering Allele Frequency** greater than expected for disease • **>.05%**	Gene/Disease-Specific
BS2	Observed in a healthy adult individual for a dominant (heterozygous) disorder with full penetrance expected at an early age. • Do not use: *ATM* has incomplete penetrance	N/A
BS3_Variable	Well-established in vitro or in vivo functional studies shows no damaging effect on protein function • **Protein**: Per detailed notes section regarding rescue and radiosensitivity assays for use as pseudo-moderate and supporting • **RNA:** Use code BP7_O_Variable	Gene-Specific
BS4	Lack of segregation in affected members of a family. Do not use: • AD Condition: Co-segregation analysis in low-penetrance genes can lead to false positive results (PMID 32773770) • AR Condition: informative instances of lack of co-segregation in A-T families are too rare to be considered for weight at this time and can also be considered for BP2 if biallelic unaffected patients are observed in an A-T family.	N/A
SUPPORTING CRITERIA		
BP1	Missense variant in gene where only LOF causes disease • Missense pathogenic variants are known for ATM	N/A
BP2_Variable	Observed *in trans* with a pathogenic variant for a fully penetrant dominant gene/disorder. • Per **Ataxia Telangiectasia PM3|BP2 table** • Do not use observations *in cis*	Disease-Specific: Strength
BP3	In-frame deletions/insertions in a repetitive region without a known function No data at this time	N/A
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product REVEL meta-predictor is approved as a sole-source computational tool with the following thresholds • Protein: REVEL score <.249 BP4 • RNA: multiple in silico predictors agree to no	General

	impact on splicing ○ Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.	
BP5	Variant found in a case with an alternate molecular basis for disease • Do not use	N/A
BP6	Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation • Do not use	N/A - discontinued
BP7 BP7_O_Variable	A synonymous (silent) variant • **BP7:** Can be used for deep intronic variants further than (but not including) +7 (donor) and -40 (acceptor) ○ Can be used in conjunction with BP4 to achieve likely benign in the absence of conflicting data ○ Is not considered a conflicting piece of evidence against a body of evidence supporting a pathogenic splice defect • **BP7_O_Variable**: Observed Lack of aberrant RNA defect with variable weight applied depending on assay quality (see text)	General

Key: **Disease-Specific:** Disease-specific modifications based on what is known about *ATM*-related diseases; **Gene-Specific:** Gene-specific modifications based on what is known about *ATM*; **Strength:** Increasing or decreasing strength of criteria based on the amount of evidence; **N/A:** not applicable for *ATM*; **General:** Using the baseline ACMG guideline but may also expounding on some of the details.

VERY STRONG EVIDENCE OF PATHOGENICITY

PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease
Caveats:

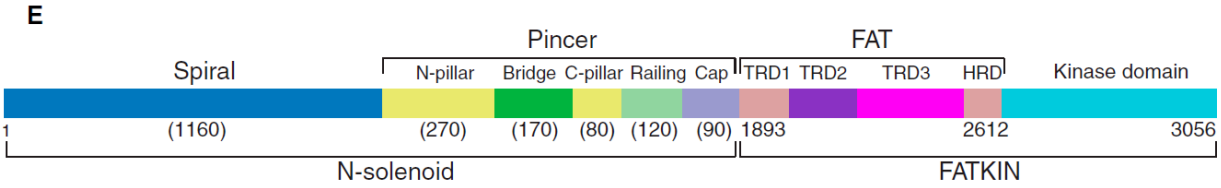
1. Use caution interpreting LOF variants at the extreme 3' end of a gene
2. Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact

Notes

1. The default RefSeq transcript for nucleotide (c.) annotation is **NM_000051.3/ENST00000278616.8.** All exons from this transcript can be considered constitutive exons without major alternate splice isoforms that could potentially rescue presumed LoF events (ENIGMA unpublished data).

- Of note, ATM is occasionally annotated with multiple non-coding first exons so exon numbering must be carefully reviewed for variant interpretation using literature sources of data.

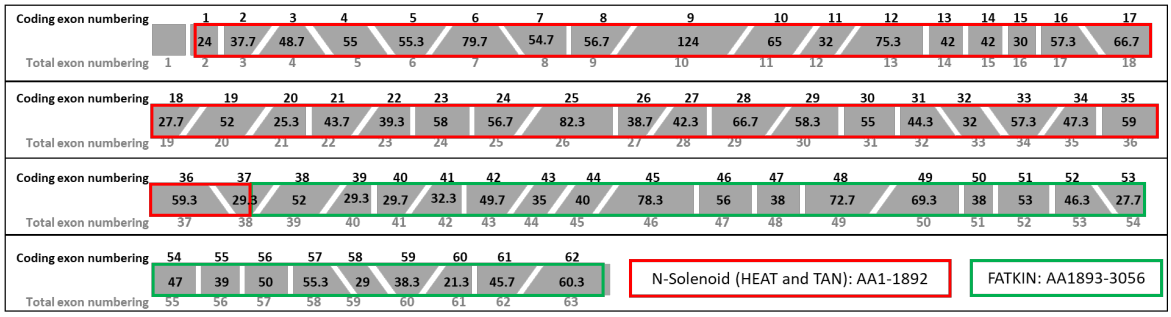
2. **The FAT/PI3K/FATC (collectively the FATKIN)** domains are considered *critical* for ATM protein function (PMID 28508030, 31740029, 31320732). PVS1 alterations that are predicted to escape NMD, but that adversely affect these domains can be granted PVS1 (as opposed to PVS1_Strong as the recommended base-line (PMID 30192042).
3. **The HEAT repeat domain** is considered *important* for protein function based on the appearance of many A-T affected individuals harboring a variant resulting in an in-frame, single exon loss in this domain (PMID 10980530, 19535770, 30819809, 15054841, 22927201, 19691550, 10330348, 17124347, 8845835, 16266405, 9463314, 24090759, 22213089). PVS1-eligible alterations that are predicted to escape NMD, but that adversely affect the HEAT repeat domain can be granted PVS1_Strong. They are limited to strong due to a lack of known missense pathogenic alterations in this domain.
4. The most 3'/C-Terminal residue considered to be pathogenic is p.R3047 (PMIDs: 8755918, 19691550, 18560558, 10980530, 26628246)
5. Below is the domain structure as annotated in PMID 28508083 and used in this body of work to delineate PVS1 boundaries



***ATM* Exon Map:** Use for Single- and Multi-Cassette Exon Losses and functional domain determination

- Number above exon in black text represents the coding exon number
- Number below exon in gray text represents total exon numbering
- The first exon is a non-coding 5'UTR-only exon
- Number within the exon represents the exon length (amino acid)
- Overhang on top: a two-nt overhang
- Overhang on bottom: a one-nt overhang
- Parallel lines represent in-frame changes (e.g. total EX6_7del is in-frame [5' of exon 6 and 3' of exon 7 are parallel]; however EX6_8del is out-of-frame [5' of exon 6 and 3' of exon 8 are not parallel)

ATM NM_000051.3/ENST00000278616.8



NOTE: Many diagrams for ATM show the FAT, PI3-K and FATC domains as separated by spacers, however these are not empirically derived and there is evidence of missense pathogenic alterations in the 'spacer' regions. This VCEP considers them a contiguous domain (PMID 28508083).

PVS1 can be applied as per the below decision tree.

PVS1_O_Variable shall be used for observed splice defects, whether from canonical +/-1,2 positions or other spliceogenic regions (including mid-exonic missense/synonymous variants that cause splice defects) with baseline weight as per the below decision tree. Weight can be further modified based on the quality of the RNA study including consideration of concepts such as:

- Starting material (where patient material is preferable to in vitro minigene)
- Use of NMD inhibitors (where use of NMD inhibitors is critical in assays using cells vs. blood)
- Primer design (to make sure it's comprehensive to capture possible multicassette events)
- Method of quantification
    - where e.g. capillary electrophoresis is preferable to estimation by gel band density
    - where SNP analysis is most preferred (where analysis of exonic SNPs and their relative presence in aberrant and WT transcripts is informative)
- Quantification (where complete effects should have increased weight over incomplete effects)

Specific guidance on the use of RNA evidence in variant assessment is not a gene-specific consideration for *ATM* at this time, therefore discretion is left to assessors until further guidance is provided for this general concept from the Sequence Variant Interpretation group.

ATM PVS1/PVS1_O Guide (Adapted from PMID 30192042)

1. PVS1 decision tree, based on ACMG/AMP rationale (Tayoun et al, 2018), introducing some code strength modifications (upgrades and downgrades, color coded as indicated), and a few instances not considered by Tayoun et al (**e.g. splice sites in non-coding exons,**. Color coded as indicated)

2. We have considered NM_000051 the clinically relevant reference transcript (63 exons, 62 coding exons, start codon located in total exon 2, coding a 3056aa protein)

3. We are not aware of any potential rescue transcripts (i.e. for the sake of simplicity, in the decision tree we will not refer to "exon is absent from biologically-relevant transcripts")

4. We define two clinically relevant domains: (i) an N-Solenoid (containing TAN and HEAT repeat domains) spaning residues 1-1892 (coded by total exons 2 to 38), and (ii) a C-terminal FATKIN domain spanning residues 1893-3056 (coded by total exons 38 to 63).

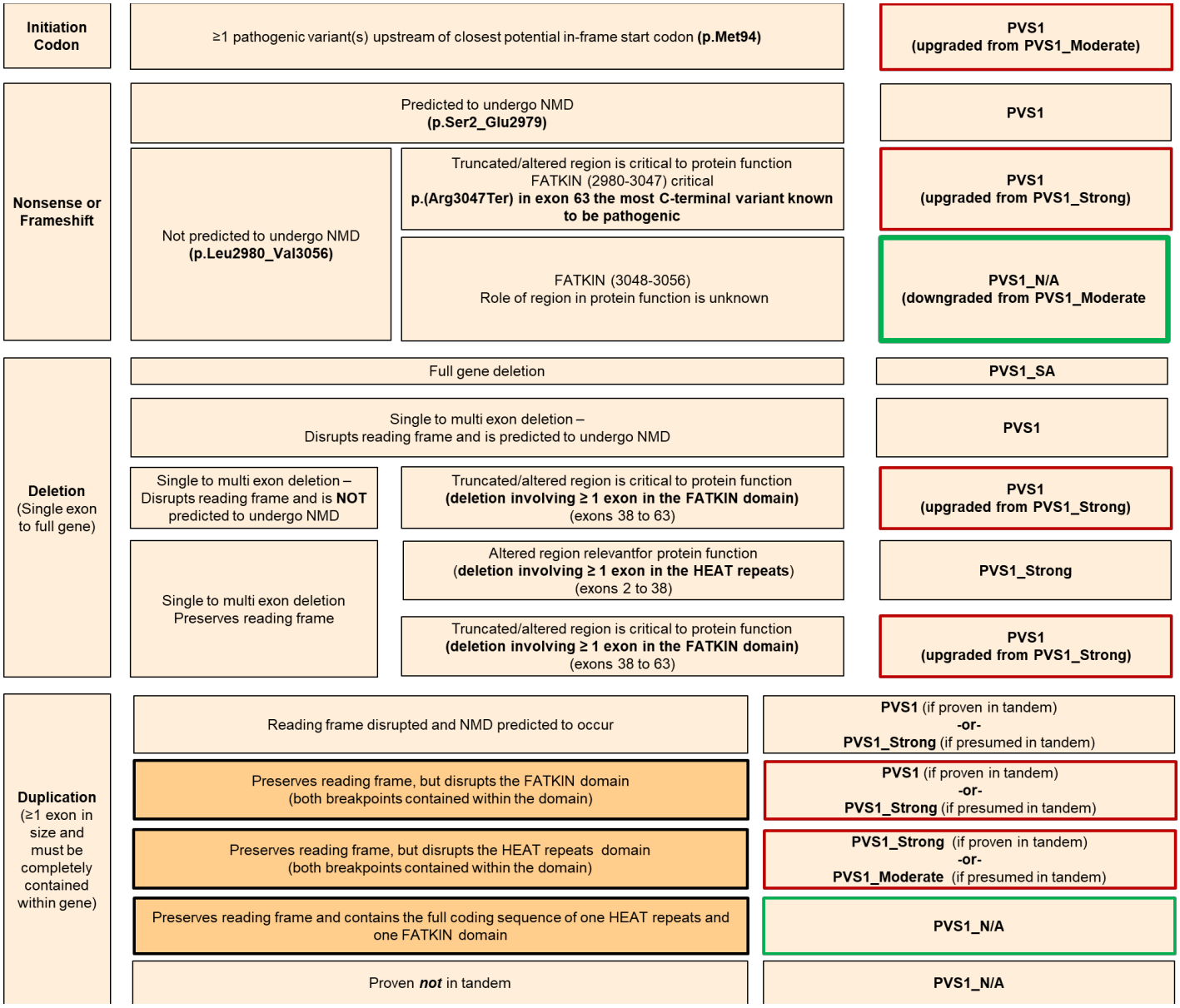
5. Based on clinical and structural data, we have considered in-frame alterations targeting HEAT repeats as **PVS1_Strong,** the only exception being any very small in-frame alterations with PROVEAN score sugesting pathogenic, that were considered **PVS1_Supporting**

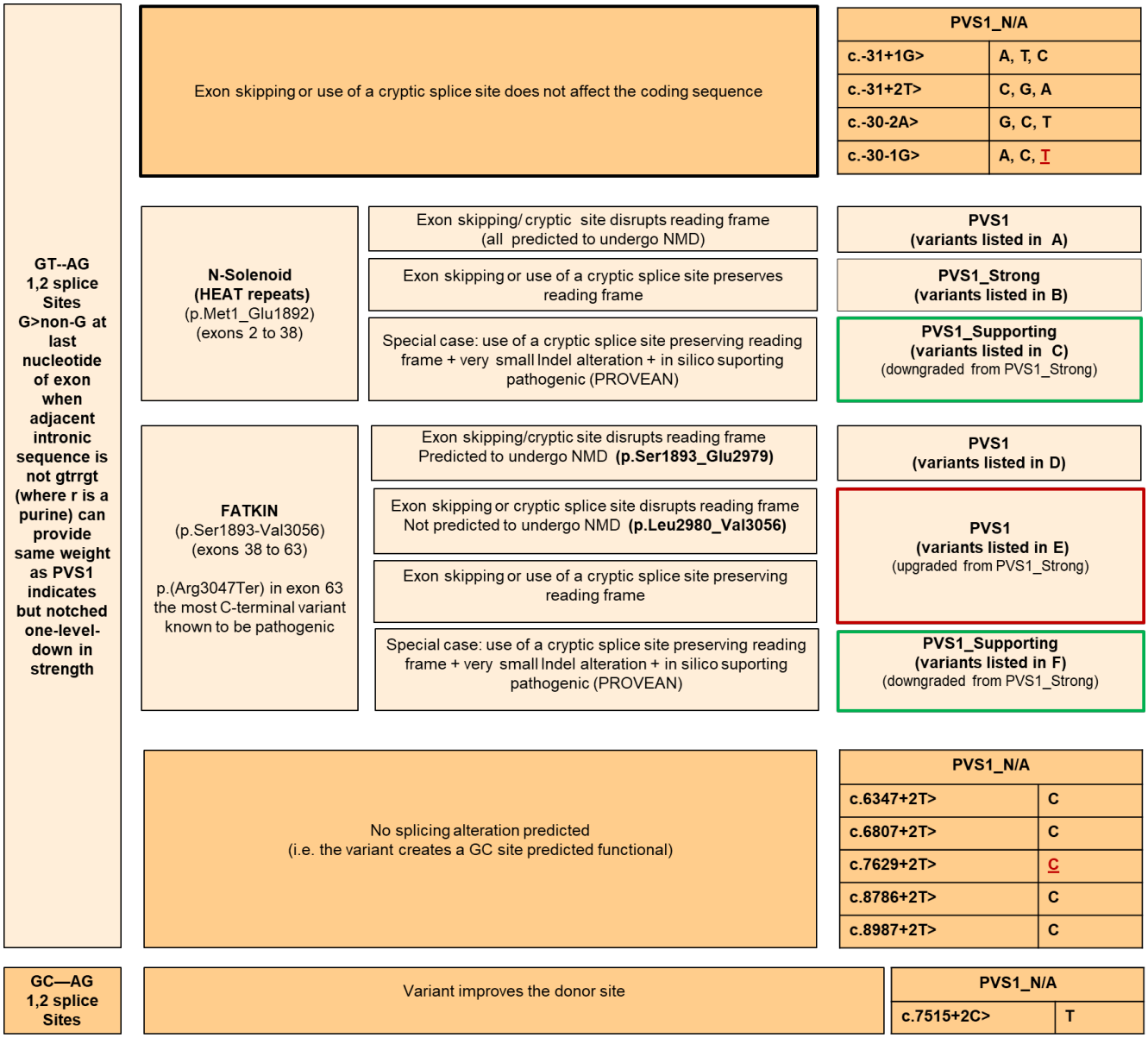
6. Based on clinical and structural data, we have considered in-frame alterations targeting FATKIN as **PVS1**, the only exception being very small in-frame alterations with PROVEAN score sugesting pathogenic, that were considered **PVS1_Supporting**

7. As far as we know, p.Arg3047Ter is the last PTC variant known to be pathogenic

8. The existence of experimental data (literature and/or personal communication from HBOP VCEP members) supporting the PVS1 weight are denoted by **red-underline** in the PVS1 decision tree.

e


Initiation Codon
≥1 pathogenic variant(s) upstream of closest potential in-frame start codon (p.Met94)
PVS1 (upgraded from PVS1_Moderate)
Nonsense or Frameshift
Predicted to undergo NMD (p.Ser2_Glu2979)
PVS1
Not predicted to undergo NMD (p.Leu2980_Val3056)
Truncated/altered region is critical to protein function FATKIN (2980-3047) critical p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic
PVS1 (upgraded from PVS1_Strong)
FATKIN (3048-3056) Role of region in protein function is unknown
PVS1_N/A (downgraded from PVS1_Moderate
Deletion (Single exon to full gene)
Full gene deletion
PVS1_SA
Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD
PVS1
Single to multi exon deletion – Disrupts reading frame and is NOT predicted to undergo NMD
Truncated/altered region is critical to protein function (deletion involving ≥ 1 exon in the FATKIN domain) (exons 38 to 63)
PVS1 (upgraded from PVS1_Strong)
Single to multi exon deletion Preserves reading frame
Altered region relevantfor protein function (deletion involving ≥ 1 exon in the HEAT repeats) (exons 2 to 38)
PVS1_Strong
Truncated/altered region is critical to protein function (deletion involving ≥ 1 exon in the FATKIN domain) (exons 38 to 63)
PVS1 (upgraded from PVS1_Strong)
Duplication (≥1 exon in size and must be completely contained within gene)
Reading frame disrupted and NMD predicted to occur
PVS1 (if proven in tandem) -or- PVS1_Strong (if presumed in tandem)
Preserves reading frame, but disrupts the FATKIN domain (both breakpoints contained within the domain)
PVS1 (if proven in tandem) -or- PVS1_Strong (if presumed in tandem)
Preserves reading frame, but disrupts the HEAT repeats domain (both breakpoints contained within the domain)
PVS1_Strong (if proven in tandem) -or- PVS1_Moderate (if presumed in tandem)
Preserves reading frame and contains the full coding sequence of one HEAT repeats and one FATKIN domain
PVS1_N/A
Proven *not* in tandem
PVS1_N/A


GT--AG 1,2 splice Sites G>non-G at last nucleotide of exon when adjacent intronic sequence is not gtrrgt (where r is a purine) can provide same weight as PVS1 indicates but notched one-level-down in strength
Exon skipping or use of a cryptic splice site does not affect the coding sequence
PVS1_N/A
c.-31+1G> A, T, C
c.-31+2T> C, G, A
c.-30-2A> G, C, T
c.-30-1G> A, C, T
N-Solenoid (HEAT repeats) (p.Met1_Glu1892) (exons 2 to 38)
Exon skipping/ cryptic site disrupts reading frame (all predicted to undergo NMD)
PVS1 (variants listed in A)
Exon skipping or use of a cryptic splice site preserves reading frame
PVS1_Strong (variants listed in B)
Special case: use of a cryptic splice site preserving reading frame + very small Indel alteration + in silico suporting pathogenic (PROVEAN)
PVS1_Supporting (variants listed in C) (downgraded from PVS1_Strong)
FATKIN (p.Ser1893-Val3056) (exons 38 to 63)
p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic
Exon skipping/cryptic site disrupts reading frame Predicted to undergo NMD (p.Ser1893_Glu2979)
PVS1 (variants listed in D)
Exon skipping or cryptic splice site disrupts reading frame Not predicted to undergo NMD (p.Leu2980_Val3056)
PVS1 (variants listed in E) (upgraded from PVS1_Strong)
Exon skipping or use of a cryptic splice site preserving reading frame
Special case: use of a cryptic splice site preserving reading frame + very small Indel alteration + in silico suporting pathogenic (PROVEAN)
PVS1_Supporting (variants listed in F) (downgraded from PVS1_Strong)
No splicing alteration predicted (i.e. the variant creates a GC site predicted functional)
PVS1_N/A
c.6347+2T> C
c.6807+2T> C
c.7629+2T> C
c.8786+2T> C
c.8987+2T> C
GC—AG 1,2 splice Sites
Variant improves the donor site
PVS1_N/A
c.7515+2C> T

N-terminal HEAT repeats
(exon2 to exon 38)

C-terminal FATKIN
(exon 38 to exon 63)
p.(Arg3047Ter) in exon 63 the most C-terminal variant known to be pathogenic

Exon skipping or use of a cryptic splice site disrupts reading frame
(all predicted to undergo NMD)

PVS1 (list A)	
c.72+1G>	A, C, T
c.72+2T>	A, C, G
c.73-2A>	C, G, T
c.73-1G>	A, C, T
c.185+1G>	A, C, T
c.185+2T>	A, C, G
c.186-2A>	C, G, T
c.186-1G>	A, C, T
c.331+1G>	A, C, T
c.331+2T>	A, C, G
c.497-2A>	C, G, T
c.497-1G>	A, C, T
c.662+1G>	A, C, T
c.662+2T>	A, C, G
c.663-2A>	C, G, T
c.663-1G>	A, C, T
c.901+1G>	A, C, T
c.901+2T>	A, C, G
c.902-2A>	C, G, T
c.902-1G>	A, C, T
c.1065+1G>	A, C, T
c.1065+2T>	A, C, G
c.1066-2A>	C, G, T
c.1066-1G>	A, C, T
c.1235+1G>	A, C, T
c.1235+2T>	A, C, G
c.1236-2A>	C, G, T
c.1236-1G>	A, C, T
c.1803-2A>	C,G,T
c.1803-1G>	A,C,T
c.1899-2A>	C, G, T
c.1899-1G>	A, C, T
c.2124+1G>	A,C,T
c.2124+2T>	A,C,G
c.2125-2A>	C, G, T
c.2125-1G>	A, C, T
c.2251-2A>	C, G, T
c.2251-1G>	A, C, T

PVS1 (list A)	
c.2467-2A>	G
c.2467-1G>	A
c.2638+1G>	A, C, T
c.2638+2T>	A, C, G
c.2639-2A>	C, G, T
c.2639-1G>	A, C, T
c.2838+1G>	A, C, T
c.2838+2T>	A, C, G
c.2921+1G>	A, C, T
c.2921+2T>	A, C, G
c.2922-2A>	C, G, T
c.2922-1G>	A, C, T
c.3077+1G>	A, C, T
c.3077+2T>	A, C, G
c.3078-2A>	C, G, T
c.3078-1G>	A, C, T
c.3153+1G>	A, C, T
c.3153+2T>	A, C, G
c.3154-2A>	C, G, T
c.3154-1G>	A, C, T
c.3284+1G>	A, C, T
c.3284+2T>	A, C, G
c.3285-2A>	C, G, T
c.3285-1G>	A, C, T
c.3402+1G>	A, C, T
c.3402+2T>	A, C, G
c.3403-2A>	C, G, T
c.3403-1G>	A, C, T
c.3577-2A>	C, G, T
c.3577-1G>	A, C, T
c.3746+1G>	A, C, T
c.3746+2T>	A, C, G
c.3747-2A>	C, G, T
c.3747-1G>	A, C, T
c.3994-2A>	C, G, T
c.3994-1G>	A, C, T
c.4109+1G>	A, C, T
c.4109+2T>	A, C, G

PVS1 (list A)	
c.4110-2A>	C, G, T
c.4110-1G>	A, C, T
c.4236+1G>	A, C, T
c.4236+2T>	A, C, G
c.4237-1G>	A
c.4436+1G>	A, C, T
c.4436+2T>	A, C, G
c.4437-1G>	A
c.4611+1G>	A, C, T
c.4611+2T>	A, C, G
c.4777-2A>	C, G, T
c.4777-1G>	A, C, T
c.4909+1G>	A, C, T
c.4909+2T>	A, C, G
c.5006-2A>	C, G, T
c.5006-1G>	A, C, T
c.5177+1G>	A, C, T
c.5177+2T>	A, C, G
c.5178-2A>	C, G, T
c.5178-1G>	A, C, T
c.5319+1G>	A, C, T
c.5319+2T>	A, C, G
c.5320-2A>	C, G, T
c.5320-1G>	A, C, T
c.5496+2T>	A, C, G
c.5497-2A>	C, G, T
c.5497-1G>	A, C, T
c.5674+1G>	A, C, T
c.5674+2T>	A, C, G
c.5675-2A>	G
c.5675-1G>	A, C, T
c.5762+1G>	A, C, T
c.5762+2T>	A, C, G

PVS1 (list D)	
c.5674+2T>	A, C, G
c.5675-2A>	G
c.5675-1G>	A, C, T
c.5762+1G>	A, C, T
c.5763-2A>	C, G, T
c.5763-1G>	A, C, T
c.6006+1G>	A, C, T
c.6006+2T>	A, C, G
c.6007-2A>	C, G, T
c.6007-1G>	A, C, T
c.6095+1G>	A, C, T
c.6095+2T>	A, C, G
c.6096-2A>	C, G, T
c.6096-1G>	A, C, T
c.6198+1G>	A, C, T
c.6198+2T>	A, C, G
c.6199-1G>	A
c.6347+1G>	A, C, T
c.6347+2T>	A, G
c.6453-2A>	C, G, T
c.6453-1G>	A, C, T
c.6573-2A>	C, G, T
c.6573-1G>	A, C, T
c.6807+1G>	A, C, T
c.6807+2T>	A, G
c.7090-2A>	C, G, T
c.7090-1G>	A, C, T
c.7307+1G>	A, C, T
c.7307+2T>	A, C, G
c.7308-2A>	C, G, T
c.7308-1G>	A, C, T
c.7515+1G>	A, C, T
c.7515+2C>	A, G
c.7516-2A>	C, G, T
c.7516-1G>	A, C, T
c.7789-2A>	C, G, T
c.7789-1G>	A, C, T
c.7927+1G>	A, C, T
c.7927+2T>	A, C, G

PVS1 (list D)	
c.8010+1G>	A, C, T
c.8010+2T>	A, C, G
c.8011-2A>	C, G, T
c.8011-1G>	A, C, T
c.8152-2A>	G
c.8152-1G>	A
c.8419-2A>	G
c.8419-1G>	A
c.8584+1G>	A, C, T
c.8584+2T>	A, C, G
c.8672-2A>	C, G, T
c.8672-1G>	A, C, T
c.8786+1G>	A, C, T
c.8786+2T>	A, G
c.8787-2A>	C, G, T
c.8787-1G>	A, C, T
c.8850+1G>	A, C, T
c.8850+2T>	A, C, G
c.8851-1G>	A

Related publication(s): Date Approved: January 19, 2022

N-terminal HEAT repeats
(exons 2 to 38)

Exon skipping or use of a cryptic splice site preserves reading frame

PVS1_Strong (list B)	
c.332-2A>	C, G, T
c.332-1G>	A, C, T
c.496+1G>	A, C, T
c.496+2T>	A, C, G
c.1607+1G>	A, C, T
c.1607+2T>	A,C,G
c.1608-2A	C,G,T
c.1608-1G>	A,C,T
c.1802+1G>	A, C, T
c.1802+2T>	A, C, G
c.1898+1G>	A, C, T
c.1898+2T>	A, C, G
c.2250+1G>	A, C, T
c.2250+2T>	A, C, G
c.2376+1G>	A, C, T
c.2376+2T>	A, C, G
c.2377-2A>	C, G, T
c.2377-1G>	A, C, T
c.2466+1G>	A, C, T
c.2466+2T>	A, C, G
c.3576+1G>	A, C, T
c.3576+2T>	A, C, G
c.3993+1G>	A, C, T
c.3993+2T>	A, C, G
c.4612-2A>	C, G, T
c.4612-1G>	A, C, T
c.4776+1G>	A, C, T
c.4776+2T>	A, C, G

PVS1_Strong (list B)	
c.4910-2A>	C, G, T
c.4910-1G>	A, C, T
c.5005+1G>	A, C, T
c.5005+2T>	A, C, G
c.5496+1G>	A, C, T

Very small Indel predicted damaging by PROVEAN

PVS1_Supporting (list C)		
		PROVEAN score
c.2467-2A>	C,T	-8.91
c.2467-1G>	C,T	
c.2839-2A>	C,G,T	-17.71
c.2839-1G>	A,C,T	
c.4237-2A>	C, G, T	-19.00
c.4237-1G>	C, T	
c.4437-2A>	C, G, T	-20.08
c.4437-1G>	C, T	
c.5675-2A	C, T	-4.98

C-terminal FATKIN
(exon 38 to 63)

Exon skipping or use of a cryptic splice site preserves reading frame, or PTC not predcited to undergo NMD

PVS1 (list E)	
c.5918+1G>	A, C, T
c.5918+2T>	A, C, G
c.5919-2A>	C, G, T
c.5919-1G>	A, C, T
c.6348-2A>	C, G, T
c.6348-1G>	A, C, T
c.6452+1G>	A, C, T
c.6452+2T>	A, C, G
c.6572+1G>	A, C, T
c.6572+2T>	A, C, G
c.6808-2A>	C, G, T
c.6808-1G>	A, C, T
c.6975+1G>	A, C, T
c.6975+2T>	A, C, G
c.6976-2A>	C, G, T
c.6976-1G>	A, C, T
c.7089+1G>	A, C, T
c.7089+2T>	A, C, G
c.7629+1G>	A, C, T
c.7629+2T>	A, G
c.7630-2A>	C, G, T
c.7630-1G>	A, C, T
c.7788+1G>	A, C, T
c.7788+2T>	A, C, G
c.8151+1G>	A, C, T
c.8151+2T>	A, C, G

PVS1 (list E)	
c.8268+1G>	A, C, T
c.8268+2T>	A, C, G
c.8269-1G>	A
c.8418+1G>	A, C, T
c.8418+2T>	A, C, G
c.8585-2A>	C, G, T
c.8585-1G>	A, C, T
c.8671+1G>	A, C, T
c.8671+2T>	A, C, G
c.8851-2A>	C, G, T
c.8851-1G>	C, T
c.8987+1G>	A, C, T
c.8987+2T>	A, G
c.8988-2A>	C, G, T
c.8988-1G>	A, C, T

Very small Indel predicted damaging by PROVEAN

PVS1_Supporting (list F)		
		PROVEAN score
c.6199-2A>	C, G, T	-14.76
c.6199-1G>	C, T	
c.7928-2A>	C, G, T	-6.13
c.7928-1G>	A, C, T	
c.8152-2A>	C, T	-73.69
c.8152-1G>	C, T	
c.8269-2A>	C, G, T	-34.54
c.8269-1G>	C, T	
c.8419-2A>	C, T	-6.32
c.8419-1G>	C, T	

Related publication(s): Date Approved: January 19, 2022

STRONG EVIDENCE OF PATHOGENICITY

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

- **Protein:** This rule may be applied only when a splice defect is ruled out for both alterations either by RNA analysis and/or *in silico* splice predictions
- **RNA**: This rule can be applied as **PS1_ RNA_Moderate** if a known pathogenic alteration has a confirmed splice defect (application of PVS1_O_Variable) and the alteration under investigation is predicted to have the same splice defect to similar or greater magnitude (worse predictions under PP3_RNA).
    - The variant used to apply PS1_RNA_Moderate variant must be approved by the HBOP VCEP as LP or P

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, *etc.* can contribute to non-maternity

- Autosomal Dominant Disease: Do not use-Informative *de novo* occurrences have not yet been observed for autosomal dominant disease. As breast cancer is relatively common and occurs frequently as an apparently sporadic event, *de novo* is unlikely to ever be informative unless specific features of *ATM*-related-breast cancer are identified.
- Autosomal Recessive Disease: Do not use- *de novo* occurrences are too rare to be informative at this time. In addition, in a biallelic state have an exceedingly low probability of being able to be confirmed as *in trans* because parental testing (and identification of one variant in each parent) is typically required without the use of long-range technologies, which is a particular challenge for very large genes such as *ATM.*

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product.

NOTE: Do not use phenotypic evidence (e.g. a lack of ATM activity in cells from an Ataxia Telangiectasia patient) as functional data. That is a general assay that confirms the patient's diagnosis and should be considered as part of PM3. However, splice data from patient material *can* be considered a functional effect because the effect is relatively specific to the variant (an undetected *ATM* variant is unlikely to cause the same splice defect as the variant under consideration for splice defect). See the accompanying Supplementary Tables 1 and 2 for details on three papers using the below methods.

Protein functional studies (See Supplementary Tables 1 and 2)

- **PS3_Moderate**: A-T (ATM null cell line) failure-to-rescue studies (typically target phosphorylation) PLUS confirmatory radiosensitivity assay;
- **PS3_Supporting**: A-T (ATM null cell line) rescue study only;
- **No Weight**: radiosensitivty only (non-specific)

RNA functional studies shall be coded as PVS1_O (where O is for 'Observed')

- Please see PVS1_O section (above) for guidance on baseline weights and modifications of weight based on quality

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

- **PS4 Case-control studies** with OR ≥2, p≤.05 with lower 95% CI >1.5
- **PS4_Moderate:** Do not use-Proband counting for genes causing a common disorder need to be calibrated in a population-specific way before use.

MODERATE EVIDENCE OF PATHOGENICITY

PM1 Located in a mutational hot spot and/or critical and well-established functional domain (*e.g.* active site of an enzyme) without benign variation

- **Do not use**: Benign and pathogenic variants are known to occur within the same domains and germline mutational hotspots are not well defined at this time

PM2 Absent from controls (or at extremely low frequency if recessive) in GnomAD

- **PM2_Supporting**
  - Rare is considered ≤.001% in all sub-populations but only when N is >1.
    - *e.g. if a sole sub-population has a general sub-population maximum frequency >.001% but N=1, PM2_Supporting applies. If, however, N=2, do not apply PM2_Supporting.*
    - Evidence supports that most variants appearing as singletons in the general population database ExAC, remain singletons (PMID 27535533)
  - There must be sufficient coverage at the locus (>30X, PMID 33600021)
  - Is not considered a conflicting piece of evidence for variants that otherwise are likely benign/benign

PM3 For recessive disorders, detected in *trans* with a pathogenic variant

- Ataxia Telangiectasia (A-T) is a rare, severe, early-onset disease with some exceptions denoted 'variant' or 'atypical' A-T in which cases phenotypes are more mild with slower progression. Phenotypes associated with A-T are very specific and do not generally require differential diagnosis. Therefore, publications that claim a 'clinical diagnosis of A-T' are taken at face value and granted a 'confident diagnosis. Specific phenotype criteria may qualify for 'confident or 'consistent' diagnosis of A-T based on the below criteria. No additional weight modifications are made for 'atypical' cases if they meet 'confident or 'consistent' criteria as although the disease progression is different, the clinical features are the same.

Ataxia Telangiectasia PM3|BP2 table:

- **Note:** Footnote 1 indicates that variants achieving PM3 may not have a general population frequency >.01%
- **Note:** Multiple unrelated cases are additive.
  - *For example, one individual with a 'confident A-T phenotype' is homozygous for a variant scores 2.0 points. Another individual who has a 'consistent A-T phenotype' and has the*

same variant and another phase-unknown truncating ATM variant scores 1.0 points. The total points towards PM3 are 3.0 points leading to PM3 used as its baseline moderate strength.

Classification/Zygosity of other variant[1]	Points per unrelated A-T Proband (PM3)			
	Confirmed in *trans*	Phase unknown	Second variant unidentified or VUS	Homozygous
Phenotype *confident*	4.0	2.0	1.0	2.0
Phenotype *consistent*	2.0	1.0	0.5	1.0

	Points per Unaffected Adult (>18yo) Proband (BP2)		
	Confirmed in *trans*	Phase Unknown	Homozygous (max -2.0)
Pathogenic or Likely pathogenic variant in a patient	-4.0	-2.0	Laboratory Setting -2.0 Database Setting -1.0
[1]May not exceed general population frequency > .01% **Do not use observations *in cis***			

Supporting	Moderate	Strong	Very Strong
PM3_			
1.0	**2.0**	**4.0**	**8.0**
BP2_			
-1.0	**-2.0**	**≤-4.0**	**N/A**

- CONFIDENT PHENOTYPE (must include Laboratory result)
  - Presence of ≥2 Laboratory results 1-4 (see notes) -OR-
  - Presence of Clinical feature 1a or 1b **AND** presence of Laboratory result 1 or 2 -OR-
  - Presence of Clinical feature 2 or 3 **AND** Laboratory result 1 or 2
- CONSISTENT PHENOTYPE (does not require laboratory result)
  - Presence of two or more Clinical features of ataxia (1a-1e) -OR-
  - Presence of one Clinical feature 1a or 1b **AND** either Clinical feature 2 or 3

Clinical features (Neurological and MRI findings):

1. Progressive cerebellar ataxia, manifesting as:
   a. Progressive truncal/limb ataxia
   b. Cerebellar degeneration (atrophy of the frontal and posterior vermis and both hemispheres by MRI).
   c. Oculomotor apraxia (inability to follow an object across visual fields) or abnormal ocular saccades (rapid refixation from one object to another).
   d. Choreoathetosis or dystonia (involuntary movements; twisting and repetitive movements, abnormal postures).

e. Peripheral axonal neuropathy OR Anterior horn cell neuronopathy
2. Oculocutaneous telangiectasia of the conjunctivae, ears, or face.
3. Immunodeficiency (often frequent infections) and/or leukemia/lymphoma.

Laboratory Results:

1. ATM protein levels ≤ 15% of controls in patient fibroblast or lymphoblastoid cell lines. If ATM protein levels are slightly greater than 15%, the ATM kinase activity must be shown to be "negative or low or residual" (see notes).
2. Elevated serum alpha-fetoprotein (AFP) levels >65ug/L in a patient ≥ 2 years old.
3. Increased sensitivity to ionizing radiation in patient fibroblast or lymphoblastoid cell lines.
4. Presence of a 7;14 chromosomal translocation in patient peripheral blood cells (≥ 5% of cells).

Notes:

1. ATM protein levels ≤15% of control levels show >95% sensitivity and >98% specificity for diagnosing ataxia-telangiectasia (A-T). Protein levels >15% may arise due to a missense variant, a leaky splicing variant, a variant resulting in a kinase-dead protein (where protein levels may not be affected), or a diagnosis other than A-T.
2. When assigning case report criteria based solely on laboratory results (i.e., presence of TWO or more of laboratory results 1-4), there is a greater likelihood that the most specific laboratory results #1 and #2 will be available, and that there will be some clinical indication that the individual(s) has A-T.
3. When assessing homozygous or *in trans* variants (with a likely pathogenic or pathogenic ATM variant) for possible downgrade in an unaffected individual, the individual should be 18 years or older with no evidence of A-T.

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

- Do not use for in-frame deletions/insertions that are not already PVS1-eligible as no information are available to justify the application of this rule.
- This rule can be applied towards stop-loss variants as multiple A-T patients are identified carrying stop-loss variants (PMID 8845835, 10272877, 17910737)

PM5 Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

- **Do not use** for hotspot - Multiple amino acid substitutions at the same residue can be pathogenic or benign and bioinformatic tools cannot yet confidently distinguish them
- **Apply to frameshifting or truncating** variants as PM5_supporting for variants with premature termination codons upstream of p.Arg3047 which are expected to be more severe than the most C-terminal pathogenic varint p.Arg3047*

PM6 Assumed *de novo*, but without confirmation of paternity and maternity

- **Do not use**: See PS2 for justification

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

Do not use:

- AD Condition: Co-segregation analysis in lower-penetrance genes can lead to false positive results (PMID 32773770)
- AR Condition: informative instances of co-segregation in A-T families are too rare to be formally analyzed at this time, however, this VCEP supports approaching this similarly to the ITGA2B/ITGB3 and Hearing Loss VCEPs who have outlined PP1 criteria for these autosomal recessive disorders (PMIDs 33496739, 30311386)

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

Do not use: ATM does not have a specified low-rate of benign missense variation.

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

- **Protein** Analysis: Metapredictor REVEL score ≥.733
- **RNA** Analysis: Concordance of ≥2 predictors reflecting a splice defect
    - NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
    - NOTE: PP3 can be used towards an RNA impact, a protein impact or both, as applicable. However, a variant's classification should be the sum of evidence for RNA **or** protein as tallied **independently** and should not mix-and-match evidence from RNA and protein evidence bodies.
        - *Example: Do not apply PP3 for* in silico *splice predictions toward the classification of a missense variant where all other evidence points towards a pathogenic protein effect (instead apply PP3 or BP4, as applicable, for a protein predictor).*
    - **NOTE:** PP3 for splice predictions **may not** be applied in addition to PVS1 or PVS1_O codes.
    - **NOTE:** PP3 splice predictions **may** be considered conflicting for an otherwise benign protein effect.
    - **NOTE:** PP3 for a protein prediction **may** be applied in addition to any protein PS3 evidence.

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

- Do not use for AD disorder
- For AR disorder, see PM3 for specific phenotype considerations

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation

Not applicable

STAND ALONE EVIDENCE OF BENIGN IMPACT

BA1 GnomAD **Filtering Allele Frequency** is greater than expected for disorder: **0.5%**

Two independent approaches support this general frequency and .5 was selected as a round number

1. Autosomal Dominant
   - Prevalence (breast cancer): 1:8
   - Allelic Heterogeneity: 1.0
   - Genetic heterogeneity: .02
   - Penetrance: .20
     - Max Credible AF =0.625%
2. Autosomal Recessive
   - Prevalence (A-T): 1:40,000 (PMID 1961222, 3788973, 27884168, 19440741, 1467590, 15297793)
   - Allelic Heterogeneity: 1.0
   - Genetic Heterogeneity: 1.0
   - Penetrance: .90
     - Max Credible AF = 0.527%

STRONG EVIDENCE OF BENIGN IMPACT

BS1 GnomAD **Filtering Allele Frequency** is greater than expected for disorder: **.05%**

Two independent approaches support this general frequency and .05 was selected as a round number

3. Autosomal Dominant
   - Prevalence (breast cancer): 1:8
   - Allelic Heterogeneity: .10
   - Genetic heterogeneity: .02
   - Penetrance: .20
     - Max Credible AF = 0.0625%
4. Autosomal Recessive
   - Prevalence (A-T): 1:40,000 (PMID 1961222, 3788973, 27884168, 19440741, 1467590, 15297793)
   - Allelic Heterogeneity: .10
   - Genetic Heterogeneity: 1.0
   - Penetrance: .90
     - Max Credible AF = 0.0527%

BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder with full penetrance expected at an early age.
Do not use*: ATM* has reduced penetrance

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.

- See Supplementary Tables 1 and 2 for studies using the below criteria:
    - **Protein functional studies (BS3)**
        - **BS3_Moderate (Protein)**: Both radiosensitivity and ATM-null cell line rescue (usually phosphorylation of multiple substrates) are normal.
            - Note 'Moderate' does not exist in the current ACMG weights for benign but can be considered as two supporting benign lines of evidence towards final classification
        - **BS3_Supporting (Protein):** Either radiosensitivity OR ATM-null cell line rescue (usually phosphorylation of multiple substrates) are normal
        - **NOTE:** BP4 protein predictions **may** be used in conjunction with BS3 for protein effects
    - **RNA functional studies (Use BP7_O_Variable)**

BS4 Lack of segregation in affected members of a family
Do not use*: ATM* has reduced penetrance.

SUPPORTING EVIDENCE FOR BENIGN IMPACT

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease
Do not use: *ATM* has known missense pathogenic variation

BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern

- See **Ataxia Telangiectasia PM3|BP2 table** (above)

BP3 In-frame deletions/insertions in a repetitive region without a known function
Do not use: no information

BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

- **Protein** Analysis: Metapredictor REVEL score ≤.249
- **RNA** Analysis: Concordance of ≥2 predictors reflecting no predicted splice defect
    - **NOTE**: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
    - **NOTE**: BP4 for splice predictions **may not** be applied in conjunction with

BP7_O_Variable (a lack of observed RNA defect)
- **NOTE**: BP4 for protein predictors **may** be applied to BS3_Variable for protein effects.
- **NOTE**: BP4 could be used towards an RNA impact, a protein impact or both, as applicable. However, a variant's classification should be the sum of evidence for RNA or protein as tallied independently and should not mix-and-match evidence from RNA and protein evidence bodies.
  - *Example: Do not apply BP4 for* in silico *splice predictions toward the classification of a missense variant where all other evidence points towards a benign protein effect (instead apply PP3 or BP4, as applicable, for a protein predictor).*

BP5 Variant found in a case with an alternate molecular basis for disease
Do not use: Cases with multiple pathogenic variants have been observed with no noticeable difference in phenotype (e.g. BRCA1 and BRCA2). In addition, ATM has low penetrance and will naturally occur with other pathogenic variants more frequently due to higher tolerance/presence in the general population.

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation
N/A Discontinued by ACMG/AMP

BP7 A synonymous (silent) variant
- **BP7: Synonymous and deep intronic**
  - Can be used for deep intronic variants beyond (but not including) +7 (donor) and -40 (acceptor)
  - May also apply BP4 to achieve Likely Benign
  - Is not considered a conflicting piece of evidence against a body of evidence supporting a pathogenic splice defect

- **BP7_O_Variable: RNA functional studies**
  - Lack of aberrant splice defect: Please see PVS1_O section (above) for guidance on baseline weights and modifications of weight based on quality for RNA assays
    - **NOTE**: BP4 splice predictions **may not** be used in conjunction with BP7

RULES FOR COMBINING PATHOGENIC CRITERIA

Pathogenic

1. 1 Very Strong (PVS1, PVS1_O PM3_VeryStrong) AND
   a. ≥1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) OR
   b. ≥2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
   c. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) and 1 Supporting (PP1-PP5, PM3_Supporting) OR
   d. ≥2 Supporting (PP1-PP5, PM3_Supporting)
2. ≥2 Strong (PS1-PS4, PM3_Strong, PP1_Strong) OR

3. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND
    a. ≥3 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
    b. 2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR
    c. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥4 Supporting (PP1-PP5, PM3_Supporting)

Likely Pathogenic

1. 1 Very Strong (PVS1, PVS1_O , PM3_VeryStrong) AND 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
2. **1 Very Strong (PVS1, PVS1_O , PM3_VeryStrong) AND PM2_Supporting** OR
3. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND 1-2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
4. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR
5. ≥3 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
6. 2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR
7. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥4 Supporting (PP1-PP5, PM3_Supporting)

RULES FOR COMBINING BENIGN CRITERIA Benign

1. 1 Stand-Alone (BA1) OR
2. ≥2 Strong (BS1-BS4)

Likely Benign

1. 1 Strong OR
2. 1 Strong (BS1-BS4) and 1 Supporting (BP1-BP7, BS3_Supporting, BP7_O_Supporting) OR
3. ≥2 Supporting (BP1–BP7, BS3_Supporting, BP7_O_Supporting)